Is animal-based biomedical research being used in its original context?

Constança Carvalho: Centre for Philosophy of Science of the University of Lisbon, Portugal. constanca.carvalho@sapo.pt

Daniel Alves: Department of Animal Biology of Faculty of Sciences of the Faculty of Sciences of the University of Lisbon; Centre for Ecology, Evolution and Environmental Changes, Portugal.

Andrew Knight: Centre for Animal Welfare, University of Winchester, UK.

Luis Vicente: Centre for Philosophy of Science of the University of Lisbon, Portugal.

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Introduction

Since the second half of the twentieth century non-human animals have been widely used as models for researching human disorders. Historically this happened for two main reasons: a) animals are complex living systems; b) it is considered less ethically-contentious, as well as easier, quicker and cheaper to use non-human animals than humans. Their benefit for biomedical advancement is assumed even though systematic evaluations, though uncommon, suggest otherwise. It is crucial to evaluate whether animal-based biomedical research is successfully benefiting medical research – even through indirect pathways – or if it is being used merely to justify further animal based research. In this chapter we demonstrate that there is a lack of communication between animal-based research and clinical research. We discuss possible reasons for this and reflect on whether animal use in biomedical research is indeed fulfilling its primary purpose.

Humans share with the rest of the animal kingdom a long evolutionary story which explains common physiological and behavioural traits and adaptations. For example, basal ganglia, which are a set of subcortical nuclei involved in several motor functions, are present throughout vertebrate taxa, and are largely similar across species (Lee et al., 2015). Similarly, the raise of body temperature as a response to infection is shared by humans and other mammals (Nesse and Williams, 1996; Schaffner, 2006). Even poikilothermic (cold-blooded) animals like lizards tend to move to warmer places once they are ill, until their body temperature is several degrees above usual (Nesse and Williams, 1996). The relatively recent decoding of genomes had shown an impressive number of genes shared between ourselves and taxonomically distant species, such as the frog (Hellsten et al., 2010). All these similarities provided the basis for the untested assumption that animals provided good research models for human disorders.

However, we know that minimal biological changes can create great differences between species and individuals. For example, Darwin's finches comprise 14 closely related species that vary dramatically in their feeding habits, despite their biological proximity (Lack, 1947). Even amongst individuals of the same species, slight and almost undetectable differences can cause very different adaptive responses. For example, human beings with sickle cell trait may have increased protection from malaria, but risk sudden

death by hypoxia when visiting high altitudes or performing intense physical exertion (Scheinin and Wetli, 2009; Webber et al*.,* 2016) – which are safe activities for most people.

Despite individual differences, it is obvious that human beings are the best biomedical model for human disorders. However, clinical research is time consuming and can have severe ethical constraints – which is one of the main reasons why animals are widely used as models for human disorders. Recent *in vitro* developments have allowed us to create cultures of human cells and tissues (*e.g.* Wilson, Ahearne and Hopkinson, 2015; Petropolis et al*.,* 2016) which are considered superior to using animal samples for human-based research (Clemedson et al*.,* 1998; Huhtala et al*.*, 2008; Petropolis et al*.*, 2016). Nonetheless, amongst the scientific community, the main obstacle to the total replacement of animal use in biomedical research is not a desire to study cells, tissues or organs, but the desire to study entire, functioning bodily systems. This is considered necessary when objectives include understanding a drug effect in the whole organism, or trying to understand the etiology and pathogenesis of multifactorial disorders like mental disorders.

In silico techniques have been slowly addressing this issue, creating whole body simulations (*e.g.* Viceconti, Clapworthy and Jan, 2008; Viceconti, Henney and Morley-Fletcher, 2016). However, the availability of human data limits these models. For example, if a new disease arises, models may fail to accurately predict the response of the human body to the new pathogen due to lack of data. It should be noted that animal models also suffer from failure to accurately predict human responses. Despite the accepted potential of *in silico* techniques, unvalidated animal models are still commonly believed to be, so far, the best available for studying the entire, functioning human body.

Throughout the years various authors have asserted that animal research has made only poor contributions to medical progress (*e.g.* Fadali, 1996; Shapiro, 1998; Greek and Greek, 2003; Bailey, 2008), while others asserted the opposite (*e.g.* Illman, 2008; Shively and Clarkson, 2009; Perretta, 2009). Such assertions are based upon historical analysis, investigations into the development of various treatments, and critical reviews of animal model use. Historical accounts are disputed. A classic example was the discovery of the role of the pancreas in diabetes. Many claim that we owe this discovery to experiments conducted by Minkowski and von Mering with dogs in the second half of the 19th century (von Mering and Minkowski, cited in Bliss, 1982), whereas others argue that this medical breakthrough was made by Thomas Cawley 100 years earlier, while performing autopsies on patients who died from diabetes (Cawley, cited in Fadali 1996).

Investigations into the development of treatments are also controversial. A good example is the development of the poliomyelitis vaccine. Poliomyelitis is a viral disease which in 1916 had reached epidemic proportions. Some (*e.g.* Ilman, 2008) state that it was the experiments performed on mice and monkeys that allowed scientists to understand its pathogenesis and develop a vaccine. Furthermore, both poliomyelitis vaccines (Salk vaccine and Sabin vaccine) were initially grown in monkey kidney tissue (Dowdle et al., 2003), reinforcing the perception of the central role of animal experiments in the development of poliomyelitis treatment (Ilman, 2008). However, others (*e.g.* Fadali, 1996) claim that animal experiments delayed the vaccine's development. Rhesus monkeys, which provided a widely used animal model for poliomyelitis, misled scientists to believe that the virus was transmitted via the respiratory, rather than the digestive route (Dowling, cited in Bailey 2008), as earlier research on humans had suggested (for a review see Fadali, 1996). This mistake led to an erroneous clinical trial in 1937 where exposed children suffered olfactory damage (Parish, 1968). Also, the first poliomyelitis vaccines, grown on monkey kidney cells, were responsible for the exposure of millions of American citizens to simian virus 40, found in rare human cancers (Pennisi, 1997). When it comes to non-human primates (NHPs), these disputes become even more contentious, because public opinion is less supportive of the use of NHPs in research (EC, 2010). Furthermore, as technology evolves, better methods become available, and the apparent historical necessity of animal experiments becomes of less relevance. For example, vaccines that used to be developed using animal tissues, sometimes suboptimally due to poor efficiency (e.g. rubella vaccine developed through duck embryo cells and dog kidney cells) or zoonosis (e.g. the simian virus that reached humans through the first polio vaccines) are now being developed using human strains (Plotkin, 2017).

Recently more objective tools to assess the contribution of animal models to biomedical progress have arisen. Such is the case for systematic reviews, meta-analyses and citation analyses. Systematic reviews are literature reviews focused on a research question that try to identify, appraise and synthesize all high-quality research evidence relevant to that question. They are generally considered the best tool to produce evidence about the value of animal studies (Pound et al., 2004), not only because they are designed to include all relevant information, reducing drastically the potential for bias, but also because systematic reviews evaluate experimental designs through rigorous and objective peer-reviewed protocols such as the 'Animal Research: Reporting In Vivo Experiments (ARRIVE)' guidelines, which apply the scientific method itself to the task of reviewing research evidence (Kilkenny et al., 2010). A meta-analysis can go even further by also incorporating a statistical representation of all the reviewed studies.

In the last decade, the number of systematic reviews shedding light on the scientific value of animal studies has increased (*e.g.* Lucas et al., 2002; Corpet and Pierre, 2005; Macleod, O'Collins et al., 2005; Perel et al., 2007; Banwell, Sena and Macleod, 2009; Martić-Kehl et al., 2015). These systematic reviews have revealed: a) poor transferability of animal outcomes to human clinical trials (*e.g.*Perel et al., 2007); b) simultaneous occurrence of animal and clinical trials, rather than sequentially, as expected given that the animal experiments should be conducted first, to allow detection of possible toxicity (e.g Lucas et al., 2002.); and, c) significant methodological and design flaws in a clear majority of animal experiments (e.g. Martić-Kehl et al, 2015). As a consequence, the use of ARRIVE or similar guidelines have become more common, and which will hopefully lead to better protocols and reduce redundant studies. As for the poor transferability of animal outcomes to human trials, it can be argued that is either a consequence of poor experimental design, and/or the fact that animal models are not suitable models for human beings (Bailey and Taylor, 2016).

Another way to determine the value of animal studies is citation analysis, which consists of determining the frequency with which a study is cited in subsequent papers. Several authors have conducted citation analyses on published papers reporting data from animals used as models for human disorders (*e.g.* Knight, 2007; Long et al., 2014; Carvalho et al., 2016), showing that those papers received very few citations by human medical papers. Again, it can be debated whether this occurs: due to a false assumption that animal models are suitable models for human disorders, or because of methodological errors or both.

To try to address this issue, we performed a citation analysis on a small sample of papers reporting data from animals used to model two complex psychiatric disorders: attention deficit hyperactivity disorder (ADHD) and major depressive disorder (MDD).

ADHD is a chronic neurodevelopmental condition of multifactorial origin, marked by persistent inattention, hyperactivity and occasionally, impulsivity (APA, 2013). It affects 2.2% of children worldwide (Erskine et al., 2013), and it can be extremely disabling (APA, 2013). MDD is a complex psychiatric mood disorder characterized by a persistent feeling of sadness that seriously impairs normal day-to-day functioning, and may even lead to suicide (APA, 2013). Mental disorders are the leading cause of years lived with disability worldwide, and 40.5% of this burden is caused by MDD alone (WHO, 2008).

In this study we categorized the citations obtained into animal versus human studies, and determined whether or not they were studying the same disorder investigated by the animal study they were citing. This form of analysis is valuable for shedding light on whether animal based research is being used to advance human healthcare, or whether it is simply fueling further animal-based research. If animal studies are contributing to human healthcare advancements, then we would expect that 1) the citations made by human-based papers should be a substantial proportion of total citations, and 2) the citations should be made mainly by studies focused on the same disorder. Any substantial deviations would signal the possibility that animal-based research is not achieving its primary purpose.

Methods

We conducted a citation analysis as defined by Garfield and Merton (1979). Briefly, in a citation analysis

one defines target papers and conducts a search of all papers that cited these target papers. The information obtained can include the total number of citations and patterns of citation. We used a total of 50 target papers: 25 non-human animal studies on ADHD, and 25 non-human animal studies on MDD.

The ADHD papers were selected from the citation analysis database created in the study by Carvalho et al. (2016). We included all papers reporting data collected with primate models (7 papers), and randomly selected 18 papers from the remaining papers using the free online tool Research Randomizer (www.randomizer.org). These 25 studies were examined to determine: the proportion of citations each paper received by human papers focused on ADHD; by human papers focused on other subjects, by animal papers focused on ADHD; and by those focused on other subjects.

The MDD papers were obtained using PubMed to locate original articles using animal models to investigate Major Depressive Disorder (similar to the protocol used in Carvalho et al. (2016)). We searched PubMed using Medical Subject Heading search terms (MeSH terms): "Major Depressive Disorder" AND title/abstract: "animal" OR "rat" OR "mice" OR "mouse" OR "Rattus" OR " Mus" OR "pig" OR "Cavia" OR "Sus" OR "rabbit" OR "Leporidae" OR "Drosophila" OR "primate" OR"monkey" OR "Macaca" OR "macaque" OR "ape" OR "rhesus" OR "chimpanzee" OR "bonobo" OR "gorilla" OR "Pan" OR "Orang Utan" OR "Pongo" OR "gibbon" OR "Hylobates" OR "Colobus" OR "Baboon" OR "Papio" OR "Mandrillus" OR "Mandrill" OR "Cebus" OR "Cebuella" OR " Brachyteles" OR "Loris" OR "Nycticebus" OR "lemur" OR "dog" OR "Canis" OR "cat" OR "Felis" OR "drosphila".

We found 33 published papers using NHPs as models, and randomly selected seven using the same randomizing tool. We found over 1,000 published papers using other animals as models, and proceeded as above to randomly select 18 papers for the citation analysis. We recorded the number of citations each paper received from subsequent animal papers, and subsequent human papers. We similarly analyzed the aim of the citing paper (weather it was focused on the same disorder or another), both in animal papers and in medical ones.

Using Fishers's exact test (available on-line at http://www.kisnet.or.jp/nappa/software/star-e/freq/1x2.htm) we investigated whether there was a significant difference between the number of citations of the animal articles by human papers, and by animal research papers. We also verified whether there was a significant difference between the number of citations by subsequent articles addressing the same disorder, and subsequent articles addressing different topics. Differences were considered statistically significant if $p<0.05$.

Results

Regarding our ADHD sample, the 25 original animal studies were cited 660 times. As shown in Figure 1, animal studies were mainly cited by other animal research papers (315), of which 82 focused on ADHD and 233 focused on different subjects. The sample resulted in 69 citations by human medical papers, of which 30 focused on ADHD and 39 focused on different subjects. The remaining 345 citations were by review articles (198) or by papers describing different methods such as *in silico* or *in vitro* (147).

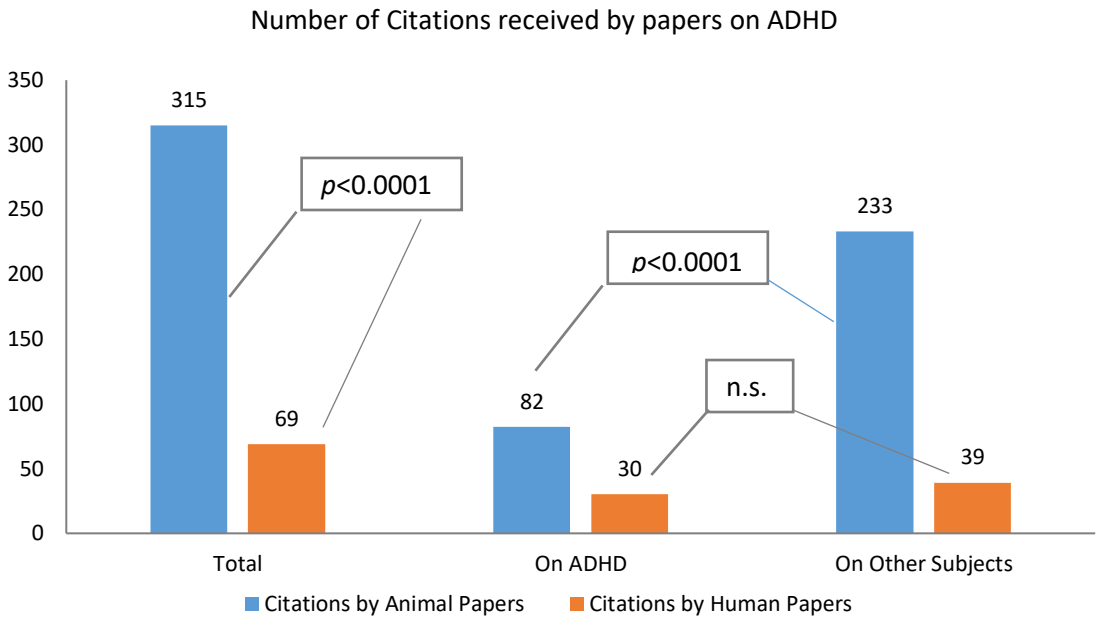

Figure 1. Citations of animal papers focused on ADHD. The columns represent the number of citations by animal research papers (blue) and human medical papers (orange) of the 25 cited papers. The total number (left), as well as the number of citations by papers studying ADHD (middle) and other subjects (right) are presented. Fisher's exact test *p*-values are also presented for each comparison made (n.s.= non-significant).

The number of citations by animal research papers was far greater than the number of citations by human medical papers (Fishers's exact test, $p<0.0001$). The number of citations by animal research papers focused on ADHD was lower than the number of citations by animal research papers focused on other subjects ($p<0.0001$). The difference between the number of citations by human medical papers on ADHD and human medical papers focused on other subjects was not statistically significant ($p=0.3355$).

The seven papers reporting NHP studies received 274 citations, 94 of which were by subsequent animal papers and 48 by human medical papers. The remaining 138 citations were by review papers (96) or by papers describing different methods such as *in silico* or *in vitro* (42). The difference between citations by animal papers and human papers was statically significant ($p=0.0001$). Of the 94 citations by subsequent animal papers, 21 were by papers focused on ADHD and 73 were by papers focused on other issues. This difference was also statistically significant ($p<0.0001$).

Of the 48 citations by human medical papers 15 were from papers focused on ADHD and 33 were from papers describing other disorders. Fisher's exact test showed that in the case of NHPs there was a statistical significant difference between the number of citations by papers on ADHD and by those focused on other subjects ($p=0.0132$).

Regarding the MDD sample, the 25 animal studies were cited 631 times. As shown in Figure 2, animal studies were mainly cited by other animal research papers (353), of which 127 focused on MDD and 226 focused on different subjects. The sample received 51 citations by human medical papers, of which 19 focused on MDD and 32 focused on different subjects. The remaining 227 citations were by review articles (163) or by papers describing different methods such as *in silico* or *in vitro* (64).

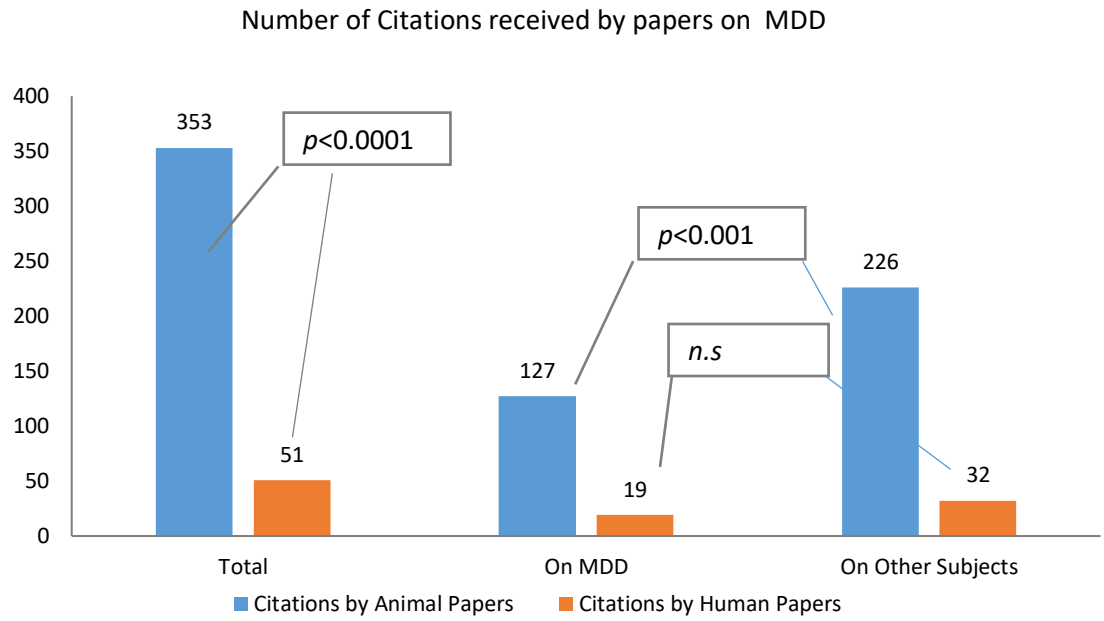

Figure 2. Citations of animal papers focused on MDD. The columns represent the number of citations by animal research papers (blue) and human medical papers (orange) of the 25 cited papers. The total number (left), as well as the number of citations by papers studying MDD (middle) and other subjects (right) are presented. Fisher's exact test *p*-values are also presented for each comparison made.

The number of citations by animal research papers was substantially greater than the number of citations by human medical papers (Fishers's exact test, $p<0.0001$). The number of citations by animal research papers focused on MDD was lower than the number of citations on focused other subjects ($p<0.0001$). The difference between the number of citations by human medical papers focused on MDD and those focused on other subjects was not statistically significant ($p=0.0919$)

The seven papers reporting NHP studies received 227 citations, 97 of which were by subsequent animal papers, and 19 of which were by human medical papers. This difference was statically significant ($p=0.001$). Of the 97 citations from subsequent animal papers, 13 were from papers on MDD, and 84 were from papers focused on other issues. This difference was statistically significant ($p<0.0001$). Of the 19 citations by human medical papers, six were from papers on MDD and 13 were from papers focused on other subjects. This difference was not statistically significant ($p=0.1670$).

Discussion

Our results suggest that animal data is mainly used by subsequent animal papers. Another trend that emerges is that papers citing animal research (whether or not they focus on human medical research) are studying disorders that differ from the one targeted in the animal study they are citing. This trend is stronger in citing papers focused on animal research.

The tendency for animal research to be more cited by subsequent animal research has been previously described (e.g. Carvalho et al., 2016). This finding contradicts the previously described assumption that citations made by human-focused papers constitute a substantial proportion of the total number of citations. Clearly, biomedical research focused on animal models does not seem to be considered important by, or particularly visible to, the human medical research community.

Our results also indicate that papers citing data collected from animal models do not necessarily target the disorder described in the animal paper. This difference appears larger in animal papers citing other animal papers, than in human medical papers that cite animal research. This contradicts the other assumption we tested: that citations should be made mainly by studies focused on the same disorder. This reinforces the

concern that animal-based research is failing to meaningfully shape healthcare advances for humans.

It can be argued that if the same animal model is used for different disorders it might be contributing more for medical research than predicted by the second assumption above. For example, DAT knock-out mice comprise a common model for ADHD, but are also used to model Parkinson or schizophrenia (Gainetdinov, 2008). Nevertheless, the citation frequency received by total human medical papers is still very low, regardless of the human medical paper's area of focus (Knight, 2007; Long et al., 2014; Carvalho et al., 2016).

The fact that animal strains are used to model several disorders may help explain the intriguing tendency for animal research papers to be cited more often in papers addressing non-related subjects than by those focused on the same disorder. This tendency was also apparent in human-based papers that cited animal-based papers focused on MDD. This may have occurred because there are 6-7 times more papers focused on MDD than on ADHD, which may mean that 25 papers were not a representative sample of MDD research. If this phenomenon was to recur with a larger sample, one could argue that this is due to the same animal strain being used for different purposes, as previously mentioned. If the strains used in MDD research are commonly used to model a greater number of disorders than strains used in ADHD research, it would be more probable that human studies focused on unrelated disorders cite studies in these strains. We did not verify this, and it should be explored in future studies.

Our data showed that even though there was no statistically significant difference between the total frequency of citations by medical papers focused on ADHD and those focused on other subjects, a bias was present regarding papers describing NHP models of ADHD. A close examination of the data allowed us to conclude that this bias was due to one paper, cited 18 times by human medical papers, 17 of which focused on disorders other than ADHD. This particular paper described the behavioral changes caused by bicuculline microinjections in external *globus pallidus*, a brain structure involved in pathogeneses of ADHD, but also in Tourette's syndrome. Most of the 18 citations by human medical papers this paper received were actually from papers related to Tourette's syndrome. If we discard this outlier, the data on NHP follows the same pattern as other ADHD papers.

Since our two assumptions have been challenged, we must discuss their causes and implications. One possible explanation for these results is that animal models only attempt to model specific symptoms or traits of complex human disorders. This oversimplification may lead to results that are non-applicable or of minimal use for human medicine. One other possible explanation is that funding is more easily attributed to studies that claim to have the potential to advance human health. This may lead animal researchers to overestimate the applicability of their projects. Another possible explanation is that communication and sharing of ideas between clinical and pre-clinical research is insufficient. Moreover, previous studies have shown that clinical and preclinical trials can occur simultaneously (Pound et al., 2004), which emphasizes this lack of communication. Although it is difficult to define an optimum communication level, this issue must be raised in both communities in order to maximize efficiency in scientific research, as well as the promotion of animal welfare. One additional possibility is that a substantial amount of animal research is needed in order to achieve a critical mass that can lead to useful breakthroughs in human health. This is a theoretical possibility that is difficult to measure and properly test. However, even if proven correct, the financial and ethical implications of this assumption should be considered. Other methods may prove to be more efficient or ethically acceptable, and this comparison could lead to a reevaluation of funding priorities. Finally, a conceivable possibility is that animal models are not suitable for biomedical research into complex human disorders. It may be possible that the uniqueness of some human disorders is just not feasibly simulated in non-human animals.

If our last suggested explanation is indeed correct, the implications must be considered. The funding currently allocated to these studies should still be available for science. While most of it would likely be redirected to other models of these disorders, some of it could be assigned either to other basic research fields or to the care of surplus animals.

Regardless of the possible explanations our results indicate that animal-based research is failing to reach human medical community, at least in the case of mental disorders like the ones we evaluated. This means that there has been a considerable financial investment and considerable suffering inflicted on the animals used for this purpose that did not translate into direct medical advances. It would be interesting to survey the practitioners working with mental disorders to assess if this is due to lack of awareness of animal-based findings, or if they consider animal-based data to be inadequate or lacking in relevance.

In conclusion, our analysis suggests that most animal-based research, at least in the case of these mental disorders, is not currently being utilized by human based researchers. Regardless of the reasons for this, the profound financial and ethical implications should lead to a re-evaluation of the current research paradigm, which is heavily reliant on invasive animal use.

References

APA (American Psychiatric Association). (2013). *Diagnostic and Statistical Manual of Mental Disorders. 5th Ed.* Arlington, VA: American Psychiatric Publishing.

Bailey, J. (2008). An Assessment of the Role of Chimpanzees in AIDS Vaccine Research. *Alternatives to Laboratory Animals*, *36(4),* pp. 381-428.

Bailey, J. and Taylor, K. (2016). Non-human Primates in Neuroscience Research: The Case Against its Scientific Necessity. *Alternatives to Laboratory Animals, 44(1), pp. 43-69.*

Banwell, V., Sena, E. and Macleod, M. (2009). Systematic review and stratified meta-analysis of the efficacy of interleukin-1 receptor antagonist in animal models of stroke. *Journal of Stroke and Cerebrovascular Diseases, 18,* pp. 269–276.

Bliss, M. (1982). *The Discovery of Insulin*: 25th Anniversary Ed. Chicago: Chigaco University Press.

Carvalho, C., Crespo, M. V., Bastos, L. F., Knight, A. and Vicente, L. (2016). Contribution of animal models to contemporary understanding of Attention Deficit Hyperactivity Disorder. *ALTEX*, 33(3), pp. 243-249.

Clemedson, C., Andersson, M., Aoki, Y., Barile, F.A., Bassi, A.M., Calleja, M.C., Castano, A., Clothier, R.H., Dierickx, P., Ekwall, B., Ferro, M., Fiskesjö, G., Garza-Ocanas, L., Gómez-Lechón, M.J., Gülden, M., Hall, T., Imai, K., Isomaa, B., Kahru, A., Kerszman, G., Kjellstrand, P., Kristen, U., Kunimoto, M., Kärenlampi, S., Lewan, L., Lilius, H., Loukianov, A., Monaco, F., Ohno, T., Persoone, G., Romert, L., Sawyer, T.W., Segner, H., Seibert, H., Shrivastava, R., Sjöström, M., Stammati, A., Tanaka, N., Thuvander, A., Torres-Alanis, O., Valentino, M., Wakuri, S., Walum, E., Wang, X.H., Wieslander, A., Zucco, F. and Ekwall, B. (1998). MEIC evaluation of acute systemic toxicity - Part IV. In vitro results from 67 toxicity assays used to test reference chemicals 31-50 and a comparative cytotoxicity analysis. *Alternatives to Laboratory Animals, 26*, pp. 131 – 183.

Corpet, D. and Pierre, F. (2005). How good are rodent models of carcinogenesis in predicting efficacy in humans? A systematic review and meta-analysis of colon chemoprevention in rats, mice and men. *European Journal of Cancer, 41,* pp. 1911-1922.

Dowdle, W., Gourville, D., Kew, O., Pallansch, M. and Wood, D. (2003). Polio eradication: the OPV paradox. *Reviews in Medical Virology, vol. 13(5)*, pp. 277-91.

EC (European Comission) (2010). Special Eurobarometer: Science and Technology Report. Available at http://www.eceae.org/no/category/watching-brief/76/eurobarometer-survey-shows-public-concern-on-animal-testing Accessed in 11 April, 2017.

Erskine, H.E., Ferrari, A.J., Nelson, P., Polanczyk, G.V., Flaxman, A.D., Vos, T., Whiteford, H.A. and Scott, J.G. (2013). Epidemiological modelling of attention-deficit/ hyperactivity disorder and conduct disorder for the Global Burden of Disease Study 2010. *Journal of Child Psychology and Psychiatry, 54,* 1263-1274.

Fadali, M. (1996). *Animal experimentation, a harvest of shame*. Los Angeles, California: Hidden Springs Press.

Garfield, E. and Merton, R. K. (1979). *Citation indexing: its theory and application in science, technology, and humanities (Vol. 8)*. New York: Wiley.

Greek, C. R. and Greek, J. S. (2003). *Sacred Cows and Golden Geese, the Human Cost of Experiments on Animals*. New York: Continuum.

Gainetdinov, R.R. (2008). Dopamine transporter mutant mice in experimental neuropharmacology. *Naunyn-Schmiedeberg's Archives of Pharmacology, 377(4-6)*, pp. 301-313.

Hellsten, U., Harland, R.M., Gilchrist, M.J., Hendrix, D., Jurka, J., Kapitonov, V., Ovcharenko, I., Putnam, N.H., Shu, S., Taher, L., Blitz, I.L., Blumberg, B., Dichmann, D.S., Dubchak, I., Amaya, E., Detter, J.C., Fletcher, R., Gerhard, D.S., Goodstein, D., Graves, T., Grigoriev, I.V., Grimwood, J., Kawashima, T., Lindquist, E., Lucas, S.M., Mead, P.E., Mitros, T., Ogino, H., Ohta, Y., Poliakov, A.V., Pollet, N., Robert, J., Salamov, A., Sater, A.K., Schmutz, J., Terry, A., Vize, P.D., Warren, W.C., Wells, D., Wills, A., Wilson, R.K., Zimmerman, L.B., Zorn, A.M., Grainger, R., Grammer, T., Khokha, M.K., Richardson, P.M. and Rokhsar, D.S. (2010). The genome of the Western clawed frog *Xenopus tropicalis*. *Science, 328(5978),* pp. 633-636.

Huhtala, A., Salminen, L., Tähti, H. and Uusitalo, H. (2008). Corneal models for the toxicity testing of drugs and drug releasing materials. *Topics in multifunctional biomaterials and devices, 1(2),* pp. 1-23.

Illman, J. (2008). *Politics, Protest and Progress: 100 years of animal research – the History of the Research Defense Society*. London: Research Defense Society.

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., and Altman, D.G. (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. *British Journal of Pharmacology, 160(7),* pp. 1577-1579.

Knight, A. (2007). The poor contribution of chimpanzee experiments to biomedical progress. *Journal of Applied Animal Welfare Science, 10,* pp. 281–308.

Lack, D. (1947). *Darwin's finches*. Cambridge: Cambridge University Press.

Lee, A.M., Tai, L.H., Zador, A. and Wilbrecht, L. (2015). Between the primate and 'reptilian'brain: rodent models demonstrate the role of corticostriatal circuits in decision making. *Neuroscience, 296,* pp. 66-74.

Long, X., Huang, J.Z. and Ho, Y.S. (2014). A historical review of classic articles in surgery field. *The American Journal of Surgery, 208(5),* pp. 841-849.

Lucas C, Criens-Poublon LJ, Cockrell CT and de Haan RJ. (2002) Wound healing in cell studies and animal model experiments by low level laser therapy; were clinical studies justified? A systematic review. *Lasers Medical Sciences, 17,* pp. 110-34.

Martić-Kehl, M.I., Wernery, J., Folkers, G. and Schubiger, P.A. (2015). Quality of Animal Experiments in Anti-Angiogenic Cancer Drug Development – A Systematic Review. *PloSone, 10(9),* pp. 1-14.

Macleod, M. R., O'Collins, T., Horky, L. L., Howells, D. W. and Donnan, G. A. (2005). Systematic review and meta-analysis of the efficacy of FK506 in experimental stroke. *Journal of Cerebral Blood Flow & Metabolism, 25,* pp. 1–9.

Nesse, R.M. and Williams, G.C. (1996). *Why we get sick: The new science of Darwinian medicine.* New York: Times Books.

Parish, H. (1968). *Victory with Vaccines. The story of immunization*. Edinburgh: E. & S. Livingstone.

Pennisi, E. (1997). Monkey virus DNA found in rare human cancers. *Science, 275*, pp. 748-749.

Perel P., Roberts, I., Sena, E., Wheble, P., Briscoe, C., Sandercock, S., Macleod, M., Mignini, L.E., Jayaram, P. and Khan, K.S. (2007). Comparison of treatment effects between animal experiments and clinical trials: systematic review. *British Medical Journal, 334,* pp. 197.

Perretta, G. (2009). Non-human primate models in neuroscience research. *Scandinavian Journal of Laboratory Animal Science, 36(1),* pp. 77-85.

Petropolis, D.B., Faust, D.M., Tolle, M., Rivière, L., Valentin, T., Neuveut, C., Hernandez-Cuevas, N., Dufour, A., Olivo-Marin, J.C. and Guillen, N. (2016). Human Liver Infection in a Dish: Easy-To-Build 3D Liver Models for Studying Microbial Infection. *PloS ONE, 11(2),* doi: 10.1371/journal.pone.0148667. eCollection 2016.

Plotkin, S. (2017). Human Cell Strains in Vaccine Development. Available at https://www.historyofvaccines.org/content/articles/human-cell-strains-vaccine-development. Accessed in 11 of April, 2017.

Pound, P., Ebrahim, S., Sandercock, P., Bracken, M. and Roberts, I (2004). Where is the evidence that animal research benefits humans? *British Medical Journal, 328,* pp. 514-7.

Schaffner. (2006). Fieber–nützliches oder schädliches, zu behandelndes Symptom? Fever–useful or noxious symptom that should be treated?. *Therapeutische Umschau, 63(3),* pp. 185-188.

Scheinin, L. and Wetli, C.V. (2009). Sudden death and sickle cell trait: medicolegal considerations and implications. *The American Journal of Forensic Medicine and Pathology, 30(2),* pp. 204-208.

Shapiro, K. (1998). *Animal models of human psychology.* Seattle: Hogrefe & Huber.

Shively, C.A. and Clarkson, T.B. (2009). The unique value of primate models in translational research. Nonhuman primate models of women's health: introduction and overview. *American Journal Primatology, 71(9),* pp. 715-721.

Viceconti, M., Clapworthy, G. and Jan, S.V.S. (2008). The Virtual Physiological Human-a European initiative for in silico human modelling. The Journal of Physiological *Sciences, 58(7),* pp. 441-446.

Viceconti, M., Henney, A. and Morley-Fletcher, E. (2016). In silico clinical trials: how computer simulation will transform the biomedical industry. *International Journal of Clinical Trials, 3(2),* pp. 37-46.

Webber, B.J., Casa, D.J., Beutler, A.I., Nye, N.S., Trueblood, W.E. and O'Connor, F.G. (2016). Preventing Exertional Death in Military Trainees: Recommendations and Treatment Algorithms From a Multidisciplinary Working Group. *Military Medicine, 181(4),* pp. 311-318.

WHO (World Health Organization). (2008). *The Global Burden of Disease: 2004 update, Table A2: Burden of Disease in DALYs by Cause, Sex and Income Group in WHO Regions, Estimates for 2004.* Geneva: World Health Organization.

Wilson, S.L., Ahearne, M. and Hopkinson, A. (2015). An overview of current techniques for ocular toxicity testing. *Toxicology, 327*, pp. 32-46.